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(54) Title: SYNTHESIS OF EPOTHILONES		
(57) Abstract		<p>Commercially feasible methods for synthesizing various epothilones precursors needed for the preparation of final epothilones are provided, including techniques for the synthesis of epothilone segment C using a stereoselective Noyori-type reduction, and the coupling of epothilone segments B and C using an aldol condensation reaction. The synthesis methods may be used to prepare naturally occurring segments as well as a large number of related analogs and homologs thereof. Final epothilones in accordance with the invention may also be of the naturally occurring variety (16-membered macrolides), while the homologs and analogs thereof are preferably up to 20-membered macrolides.</p>

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SYNTHESIS OF EPOTHILONES

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BACKGROUND OF THE INVENTION

Field of the Invention

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The present invention is broadly concerned with methods for synthesizing various epothilone segments or precursors (either naturally occurring or analogs thereof) which can be used for the efficient synthesis of complete epothilones. More particularly, the invention pertains to such synthesis methods wherein, *inter alia*, the epothilone segment C is prepared using a unique Noyori reduction scheme, and epothilone segments B and C are connected via a novel aldol condensation reaction. These syntheses can be used to prepare the naturally occurring segments and a wide variety of corresponding analogs and homologs.

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Description of the Prior Art

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The epothilones (16-membered macrolides which were initially isolated from the myxobacterium *Sorangium cellulosum*) represent a class of promising anti-tumor agents, and have been found to be potent against various cancer lines, including breast cancer cell lines. These agents have the same biological mechanism of action as Taxol, an anti-cancer drug currently used as a primary therapy for the treatment of breast cancer. Other potential applications of the epothilones could be in the treatment of Alzheimer's disease, malaria and diseases caused by gram-negative organisms. Other cancers such as ovarian, stomach, colon, head and neck and leukemia could also potentially be treated. The epothilones also may have application in the treatment of arthritis.

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In comparison to Taxol®, the epothilones have the advantage of being active against drug-resistant cell lines. Drug resistance is a major problem in chemotherapy and agents such as the epothilones have overcome this problem and hold great promise as effective agents in the fight against cancer.

30

In addition, the poor water solubility of Taxol® has led to the formulation of this drug as a 1:1 ethanol-Cremophor concentrate. It has been determined that the various hypersensitive reactions in patients such as difficulty in breathing, itchiness of the skin and low blood-pressure are caused by the oil Cremophor used in the formulation. The epothilones are more water soluble than Taxol® which has positive implications in its

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formulation. Further advantages of the epothilones include easy access to multi-gram quantities through fermentation procedures. Also the epothilones are synthetically less complex, thus structural modifications for structure activity relationship studies are easily accessible.

5 The epothilones exhibit their activity by disrupting uncontrolled cell division (mitosis), a characteristic of cancer, by binding to organelles called microtubules that are essential for this process. Microtubules play an important role in cell replication and disturbing the dynamics of this component in the cell stops cell reproduction and the growth of the tumor. Antitumor agents that act on the microtubule cytoskeleton fall
10 into two general groups: (1) a group that inhibits microtubule formation and depolymerizes microtubules and, (2) a group that promotes microtubule formation and stabilizes microtubules against depolymerization. The epothilones belong to the second group and have displayed cytotoxicity and antimitotic activity against various tumor cell lines.

15 It has been demonstrated on the basis of *in vitro* studies that the epothilones, especially epothilone B, are much more effective than Taxol® against multi-drug resistant cell line KBV-1. Preliminary *in vivo* comparisons with Taxol® in CB-17 SCID mice bearing drug-resistant human CCRF-CEM/VBL xenografts have shown that the reduction in tumor size was substantially greater with epothilone B in comparison to Taxol®.

20 In light of the great potential of the epothilones as chemotherapeutic agents, there is a need for techniques allowing the practical, large scale, economical synthesis thereof. Furthermore, there is a need for synthesis methods which facilitate the preparation of various homologs and analogs of the known epothilones, and those having affinity labels allowing study of the binding interactions of these molecules.

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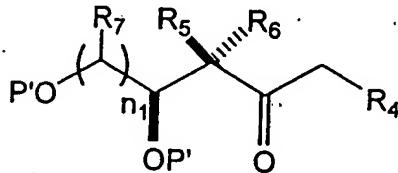
SUMMARY OF THE INVENTION

30 The present invention overcomes the problems outlined above, and provides various practical, commercially feasible synthesis routes for the production of important epothilone precursors or segments in high yield. The invention is particularly concerned with synthesis of the precursors or segments C, D (which is a combination of segments B and C) and vinyl halide epothilone precursors.

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In a first aspect of the invention, an epothilone precursor of the formula

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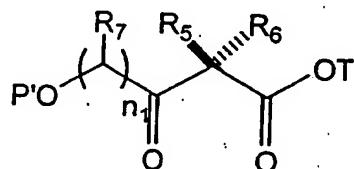


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is synthesized using a Noyori reduction reaction. In the foregoing formula, n_1 is an integer from 0-4, R_4 is selected from the group consisting of H, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1-C10 alkoxy groups, R_5 and R_6 are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1-C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R_7 is H or straight or branched chain C1-C10 alkyl groups, and P' is a protective group. The method comprises the steps of first providing a β -keto ester of the formula

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where n_1 , R_5 , R_6 , R_7 , and P' are as defined above, and T is an alkyl group. This β -keto ester is then preferentially hydrogenated at the C3 keto group to form the corresponding hydroxyester. This is accomplished by reacting the β -keto ester with a hydrogenating agent in the presence of an asymmetric organometallic molecular catalyst comprising a metal atom or ion having one or more chiral ligands coupled thereto. The synthesis is completed by then converting the hydroxyester to the epothilone precursor.

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More preferably, n_1 is an integer from 0-4, R_5 , R_6 and R_7 are each individually and respectively selected from the group consisting of H and the straight and branched chain C1-C4 lower alkyls, and the protective group is benzyl. In terms of preferred process parameters, the hydrogenating agent is preferably H_2 and the hydrogenating step is carried out at a pressure of from about 30-100 psi, more preferably 50-75 psi, and at a temperature of from about 40-100°C, more preferably from about 50-75°C. The reaction is normally allowed to proceed for a period of from about 12 hours to 5 days, and more usually for about 2-5 days. Typically, the reaction mixture is agitated during the hydrogenating step.

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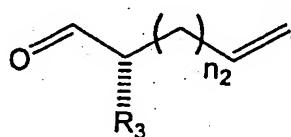
The catalyst used in the hydrogenation reaction is preferably one of the well-known Noyori catalysts such as RuBr₂(S)-binap. However, a variety of other catalysts of this type can also be employed. The catalyst is generally used at a level of from about 1-25 mol % in the reaction mixture.

In order to complete the reaction sequence, the hydroxyester resulting from the Noyori reduction is converted to the epothilone precursor segment C. A number of routes can be used to effect this conversion. Preferably, however, the conversion involves: (1) removing the P' protecting group from the hydroxyester to form a diol; (2) protecting the oxygen atoms of the diol, forming a protected diol; (3) reducing the ester function of the protected diol to a primary alcohol; (4) oxidizing the primary alcohol to the corresponding aldehyde; (5) reacting the aldehyde with a Grignard reagent having the R₄ group coupled thereto to form a secondary alcohol; and (6) oxidizing the secondary alcohol to form the final epothilone precursor.

Preferably, the P' removal step involves reacting the hydroxyester with hydrogen in the presence of a catalyst (e.g., Pd(OH)₂ or Pd/C) at a pressure of from about 40-100 psi. The oxygen atom protecting step comprises reacting the diol with TBS chloride in a compatible solvent (i.e., one that will not interfere with the desired reaction) at a temperature of from about 40-100°C for a period of from about 30-60 hours. The ester function reduction step is preferably carried out by reacting the protected diol with the reducing agent DIBAL-H at a temperature of from about -20 to -85°C. The oxidation of the primary alcohol is carried out most conveniently using 4-methylmorpholine N-oxide and a catalytic amount of tetrapropylammonium perruthenate. The Grignard reaction serving to attach the R₄ group is entirely conventional and well within the skill of the art; likewise, the final oxidation of the secondary alcohol is trivial using the aforementioned oxidation procedure, i.e., NMO and TPAP.

In another aspect of the invention, a method is provided for the production of epothilone precursor D, which is a combination of segments B and C. Segment C is of course produced as outlined above. Segment B is of the formula

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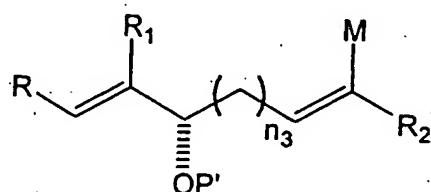
where n₂ is an integer from 1-4, and R₃ is selected from the group consisting of H, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl

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groups, and C1-C10 alkoxy groups. This segment can be efficiently produced using known techniques.

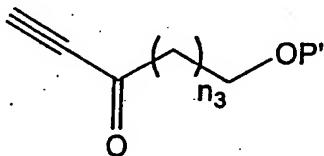
The segments B and C are connected by first reacting the segment C precursor with a base to form an enolate, followed by reacting the enolate with the segment B. These reactions are generally carried out by initially cooling the base to a temperature of about -75°C, adding the segment C precursor and elevating the temperature of the mixture to about -40°C, then recooling the mixture to at least about -75°C and adding the precursor segment B thereto.

The invention also is concerned with a method of synthesizing vinyl halide epothilone precursors having the general formula



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where n_3 is an integer from 1-4, R is selected from the group consisting of C4-C8 cycloalkyl, and substituted and unsubstituted aromatic and heteroaromatic groups, R_1 and R_2 are each individually and respectively selected from the group consisting of H; C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1-C10 alkoxy groups, P' is a protecting group, and M is either bromine or iodine. This reaction involves first providing an alkynyl ketone of the formula



25.

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wherein n_3 and P' are as previously defined. Thereafter, the alkynyl ketone is asymmetrically reduced to create the alcohol form of the alkynyl ketone. This alcohol form is then reacted with a reagent system selected from the group consisting of $(R_1)_3Al$ and zirconocene dichloride or stannyl cupration reagent and R_1 -halide to form a vinyl metal species. The vinyl metal species is then reacted with an aryl or vinyl halide to form an allyl alcohol. This allyl alcohol is then converted to the vinyl halide epothilone precursor.

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Normally, the asymmetric reduction step involves creating the reduced form of the alkynyl ketone and the resulting alcohol is protected using TBS as a protecting agent. The R₁-halide is selected from the group consisting of R₁Br and R₁I. The conversion step preferably includes the step of initially converting the allyl alcohol to an alkynyl stannane, reducing the stannane with chlorohydrido zirconocene to form a 1,1-dimetalloc Zr-Sn species. The dimetalloc species is then hydrated to form a vinyl stannane, which is then quenched with either iodine or bromine. Alternately, the conversion step may be accomplished by transmetallating the dimetalloc species with an organocuprate, quenching with an alkyl-R₂-OTf, and final quenching with either iodine or bromine incorporating the R₂ group.

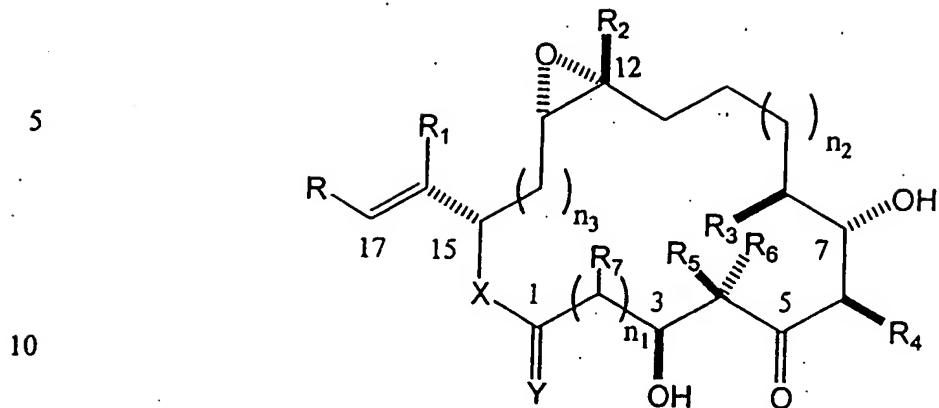
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The molecular architecture of representative epothilones (Formulae A-B) reveals three essential domains. These include the two chiral domains, namely the C1-C8 polypropionate region and the C12-C15 region, and the achiral spacer C9-C11 which unites the chiral domains. Additional structural features include a thiazole moiety, the C16 double bond, a methyl group at C4 and a *cis*-epoxide moiety (C12-C13) in the epothilones of Formula A. In the following formulae A and B, n₁ is an integer from 0-4, n₂ and n₃ are each respectively integers from 1-4, R is selected from the group consisting of C4-C8 cycloalkyl, and substituted and unsubstituted aromatic and heteroaromatic groups, R₁, R₂, R₃ and R₄ are each individually and respectively selected from the group consisting of H, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1-C10 alkoxy groups, R₅ and R₆ are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1-C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R₇ is H, or straight or branched chain C1-C10 alkyl groups, X is either oxygen or NH, and Y is either oxygen or H₂.

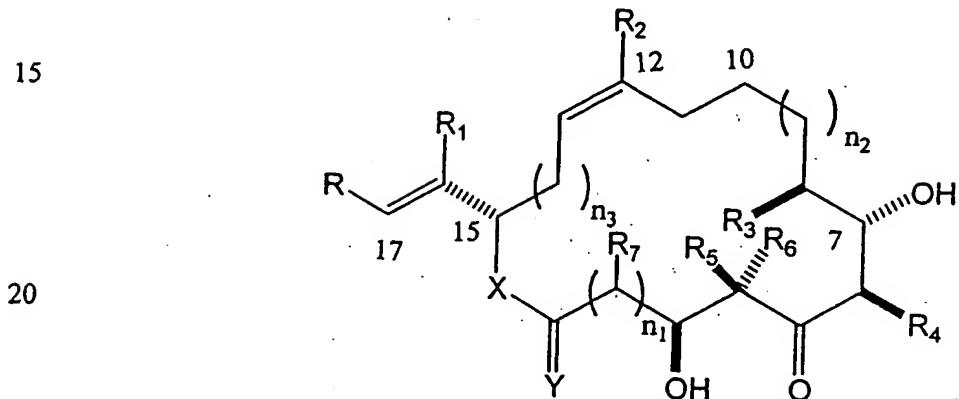
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FORMULA A



FORMULA B



25 Scheme 1 below outlines a retrosynthetic analysis respecting the total synthesis of the epothilones of Formula A in accordance with the invention, where each n_1 and n_2 equal 1, R is 2-methyl-thiazol-4-yl, R_1 is methyl, R_2 is H or methyl, R_3 , R_4 , R_5 and R_6 are methyl, R_7 is H, and X and Y are oxygen. Standard epoxidation and macrolactonization strategies are used for the formation of the C12-C13 epoxide moiety and the 16-membered macrolide. The analysis for other analog epothilones of Formula A is identical, and also for the epothilones of Formula B and its analogs, with the epoxidation step being omitted. Included among the novel features of this synthesis are the following:

30 A novel route to the C1-C6 segment labeled C in Scheme 1 that utilizes a stereoselective hydrogenation reaction, i.e., a Noyori reduction.

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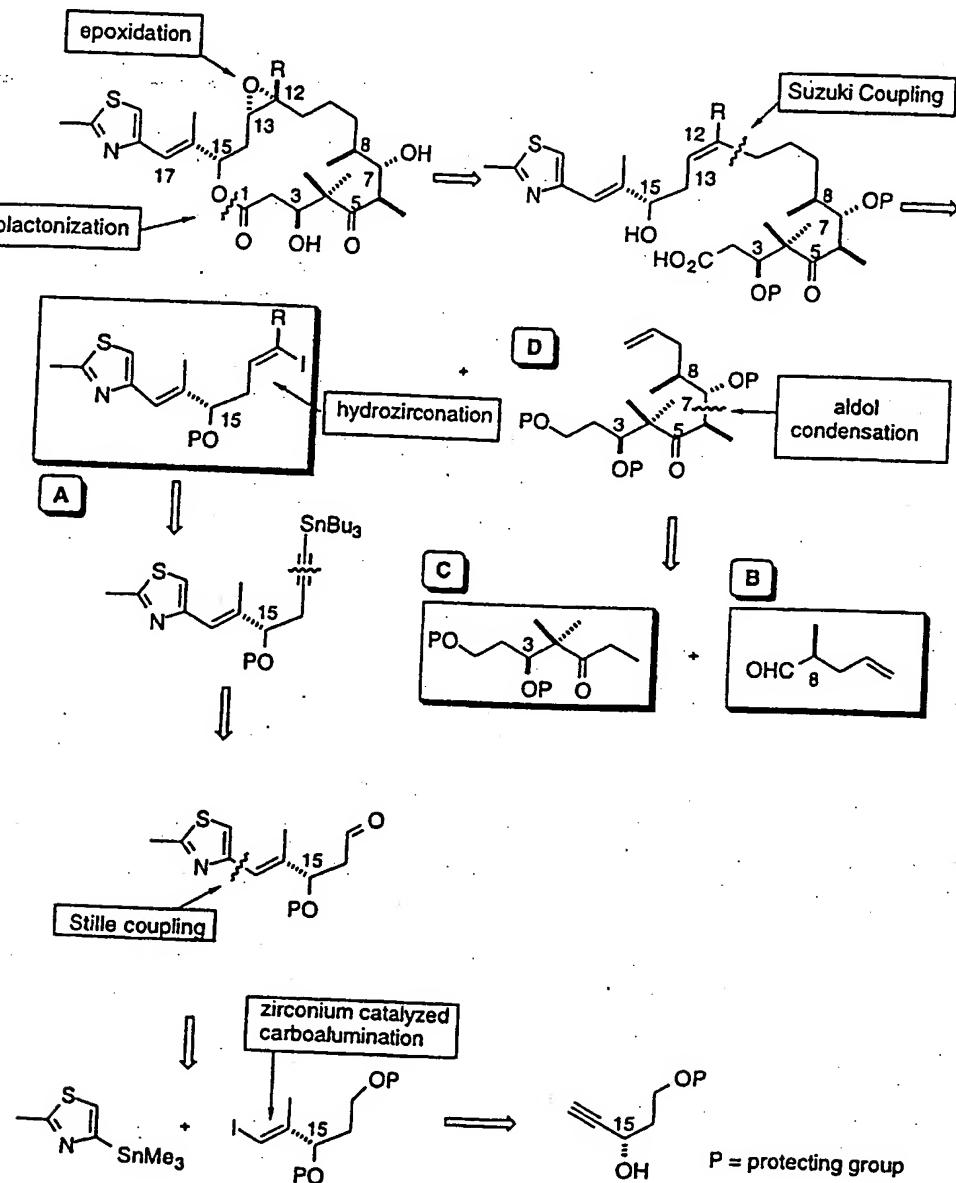
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A novel connection of segments C and the C7-C11 segment B utilizing a diastereoselective aldol reaction that renders the required diastereomer.

5 A novel route to the C12-C20 segment A which provides maximum control over the geometries of the two double bonds i.e., C12-C13 and the C16-C17 double bond. This is achieved by using stereo- and regioselective hydro/carbo-metallation reactions starting from alkyne precursors.

Scheme 1

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Synthesis of Segment C (C1-C6 of Formula A)

The synthesis of segment C has been accomplished via two unique and complementary routes, detailed in Schemes 2 and 3 below, which illustrates the synthesis of the naturally occurring segment C. A novel step in the synthesis of the C1-
5 C6 segment utilizes the Noyori hydrogenation of β -keto ester 4 to generate the requisite stereochemistry at C3. This Noyori hydrogenation (Noyori, R. et al., in *Asymmetric Hydrogenation of β -Keto Carboxylic Esters. A Practical, Purely Chemical Access to β -Hydroxy Esters in High Enantiomeric Excess*, 109 J. Am. Chem. Soc. 5856-5858 (1987)) provides the required enantiomer with high selectivities (92-95% enantiomeric
10 excess). The use of a Noyori hydrogenation reaction permits large, commercial scale production of segment C.

The required β -keto ester 4 is obtained in two steps from the readily available starting material 3-benzyloxypropionic acid (2). Asymmetric hydrogenation of 4 in methanol using RuBr₂(S)-binap as catalyst at 60 psi gives the β -hydroxyester 5 in 71-
15 85% yield (92-95% ee). Deprotection of the benzyl ether and bis-silylation of the resultant diol 6 provides ester 7. The ester is reduced to the known primary alcohol 8 using DIBAL-H. The alcohol is then oxidized to the known aldehyde 9 using a previously unreported oxidation procedure. The aldehyde is then reacted with EtMgBr using a reported procedure (Claus, et al., *Synthesis of the C1-C9 Segment of Epothilones*, Tetrahedron Lett., 38:1359-1362 (1997)) to give the known secondary alcohol 10 in 65% yield. This alcohol is then oxidized to the C1-C6 segment C using TPAP and NMO.
20

In summary, although segment C is a key synthon in previously reported total syntheses (Nicolaou, et al., *Total Syntheses of Epothilones A and B via a Macrolactonization-Based Strategy*, J. Am. Chem. Soc., 119:7974-7991 (1997)) of the epothilones, the synthetic route utilizing the asymmetric Noyori hydrogenation is unique.
25

The alternate route toward segment C allows for the introduction of affinity labels and modifications at the C4 position as shown in Scheme 3. Applying the Noyori reduction to the known unsubstituted β -keto ester 11 provides a building block that can be used for the modifications at C4 of the epothilones. This Scheme accordingly allows for modification of the epothilones and gives a more general route to introduce a variety of substituents at this position.
30

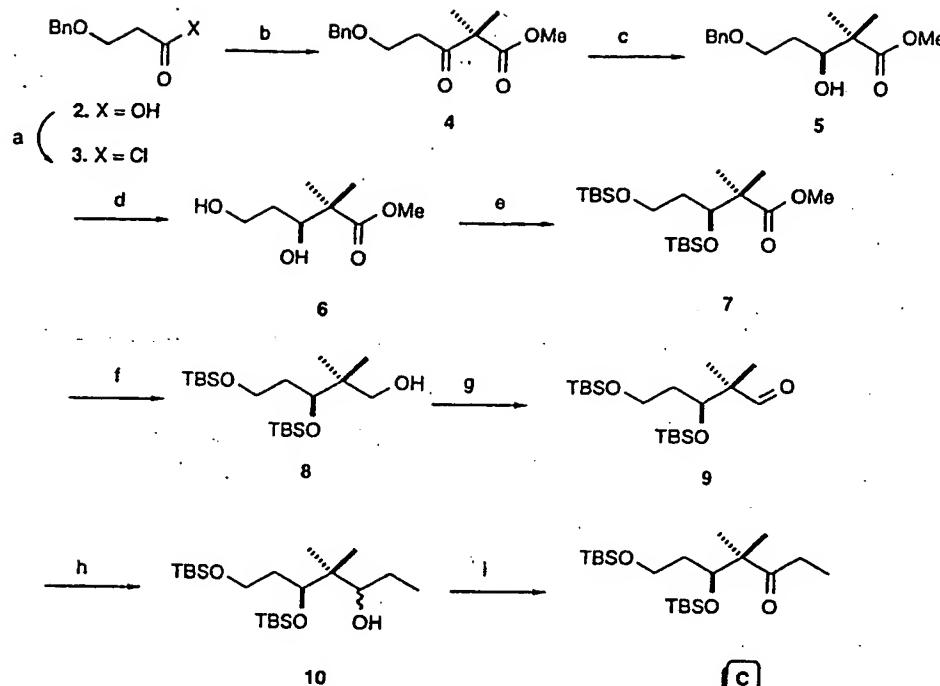
Thus, the Noyori hydrogenation of β -keto ester 11 yields the known β -hydroxy ester 12 (Ali, et al., *Formal Syntheses of Cryptophycin 1 and Arenastatin A*,
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Tetrahedron Lett., 38:1703-1706 (1997)) in 97% yield (in 97% enantiomeric excess).
The Frater alkylation of β -hydroxy ester 12 yields the previously reported α -methyl
analogue 13 (Ali, et al., *Formal Syntheses of Cryptophycin 1 and Arenastatin A*,
Tetrahedron Lett., 38:1703-1706 (1997)) in 71% yield (98% diastereomeric excess).
5 A second Frater alkylation of hydroxy ester 13 gave bis-dimethyl derivative 5 in 59%
yield which was then converted to epothilone segment C by the chemistry shown in
Scheme 2. At this stage, other substituents such as benzyl, allyl and other C1-C6 alkyl
groups can be introduced by using other electrophiles in the second Frater alkylation in
10 place of iodomethane. The novel aspect about this alternate route to segment C is the
ability to alter the substituents at the C4 position of the epothilones using the
aforementioned Frater alkylation strategy.

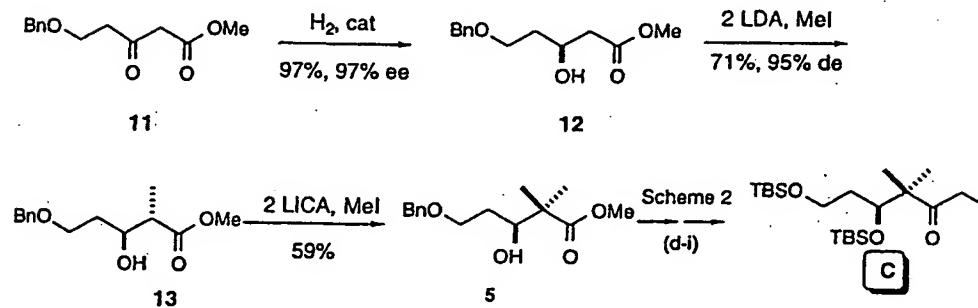
-11-

Scheme 2



a). $(COCl)_2$, CH_2Cl_2 , 99%; b) methyl isobutyrate, LICA, THF, -78 °C, 65%; c) $RuBr_2$, (*S*)-binap, H_2 , MeOH, 65 psi, 70 °C, 71%, (92% ee); d) H_2 , $Pd(OH)_2$, THF, 90%; e) TBSCl, imidazole, DMF, 60 °C, 78%; f) DIBAL-H, CH_2Cl_2 , -78 °C, 95%; g) TPAP, NMO, 4 Å MS, CH_2Cl_2 , 85%;
h) $EtMgBr$, Et_2O , -78 °C, 65%; i) same as g, 92%.

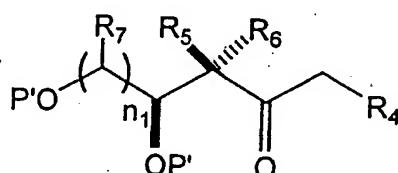
Scheme 3



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The invention makes it possible to synthesize several analogs of segment C with various chain elongations and/or substitutions at C2 and substitutions at the α -carbon relative to the keto group. It also allows for, as mentioned before, modifications at the carbon atom between the keto and the protected secondary hydroxy group with other groups. These chain extensions and substitutions are illustrated by a general Formula C, wherein n_1 is as defined above, R_5 , R_6 and R_7 are as defined previously, and P' is a protecting group, especially TBS or p-methoxybenzylidene acetal. The synthesis of these modified derivatives can be achieved utilizing chemistry exemplified in the synthesis of segment C in Schemes 2 and 3 respectively. These modified segments can then be utilized in the total synthesis of various analogs of epothilones.

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FORMULA C

Synthesis of Segment B (C7-C11 of Formula A)

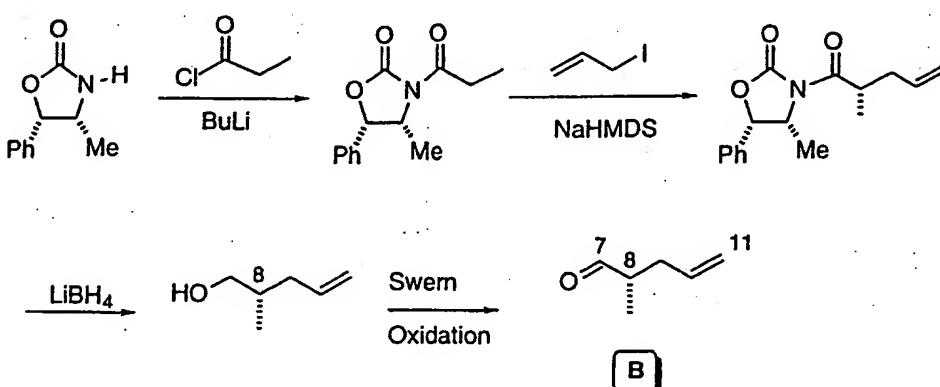
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The synthesis of the C7-C11 segment B is preferably achieved using previously reported chemistry (Lin, *Efficient Total Syntheses of Pumiliotoxins A and B, Applications of Iodide-Promoted Iminium Ion-Alkyne Cyclization in Alkaloid Construction*, J. Am. Chem. Soc., 118:9062-9072 (1996)) and is outlined in exemplary Scheme 4, which is precursor of a naturally occurring epothilone.

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Scheme 4

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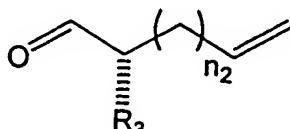


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This synthesis can also be used to introduce various chain-elongations on this segment and to introduce various other substituents at C-8. These modifications can be illustrated by Formula D, wherein n_2 and R_3 are as defined previously. Their synthesis can be achieved using chemistry exemplified in the synthesis of segment B in Scheme 4. Again, these modified segments can then be utilized in the total synthesis of various analogs of epothilones.

10



FORMULA D

Synthesis of Segment D (C1-C11 of Formula A) via Aldol Reaction

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The connection of the two segments C and B utilizes a highly diastereoselective aldol reaction, exemplified in Scheme 5 showing the connection of the two precursors B and C of a naturally occurring epothilone. When the C1-C6 ketone segment C is treated with a base, for example lithium diisopropylamide and the resultant enolate reacted with C7-C11 aldehyde segment B, a single desired diastereomer 14 was observed in 21% yield (unoptimized). This diastereoselectivity is believed to arise from a favorable nonbonding interaction between the C10-C11 double bond and the carbonyl group of the aldehyde that gives rise to the desired diastereomer. After the connection is made, the resultant secondary alcohol is protected as the corresponding *tert*-butyldimethylsilyl ether.

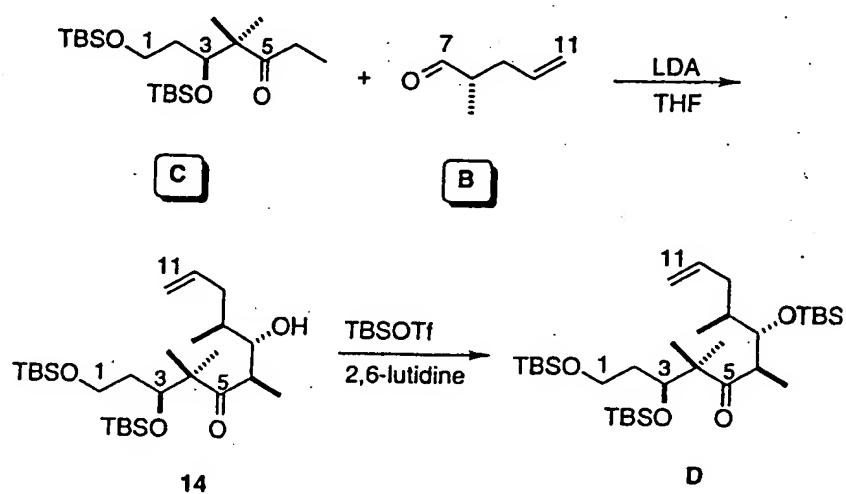
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Similar chemistries would apply for the connection of modified segments C and B of the type discussed previously and exemplified by Formulae C and D.

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Scheme 5

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Proposed Synthesis of Segment A (C12-C20 of Formula A)

The invention also provides a new route to the C12-C20 segment (segment A of the naturally occurring epothilone), and corresponding analogs thereof. This involves new ways to set the C16-C17 trisubstituted double bond and the C12-C13 *cis*-double bond, which serves as precursor to the *cis*-epoxide at C12-C13 in the epothilones.

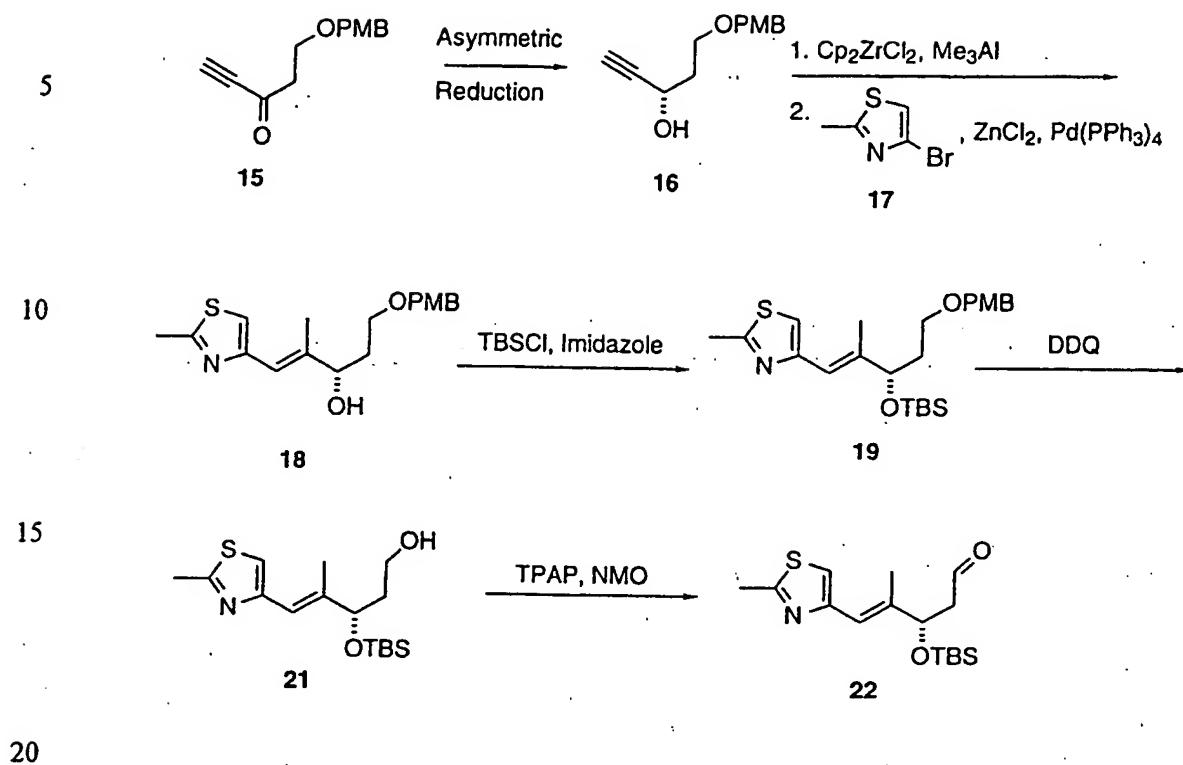
Stereoselective Construction of C16-C17 of Trisubstituted Olefin and Introduction of Thiazole in Formula A

The introduction of the thiazole moiety draws upon zirconium-catalyzed carboalumination chemistry (Wipf, *Rapid Carboalumination of Alkynes in the Presence of Water*, Agnew. Chem., Int. Ed. Engl., 32:1068-1071 (1993)) wherein a C16-C17 alkyne bond in an appropriately functionalized C13-C17 propargylic alcohol 16 (Scheme 6) is subjected to methylalumination in the presence of zirconocene dichloride (Cp_2ZrCl_2). The resultant alkenylalane is coupled with 2-methyl-4-bromothiazole 17 in the presence of zinc chloride under Pd(0) catalysis to access the trisubstituted *E*-olefin 19 stereoselectively following the protection of the alcohol 18 as the OTBS-ether.

The chiral propargylic alcohol 16 is obtained via the asymmetric reduction of the readily available alkynyl ketone 15. This is exemplified in Scheme 6, which illustrates the synthesis of the precursor for the naturally occurring epothilone. After the introduction of the thiazole moiety, the known primary alcohol 21 is revealed by deprotection of the PMB ether 19 and then oxidized to the previously reported (Mulzer, J., et al. *Easy Access to the Epothilone Family - Synthesis of Epothilone B*, Tetrahedron Lett., 39:8633-8636 (1998)), C13-C20 aldehyde 22.

-15-

Scheme 6

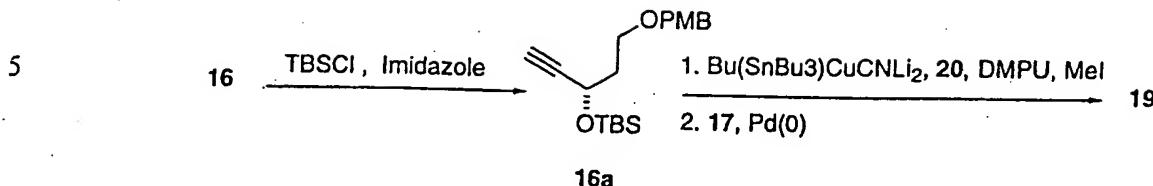


Alternately, a stannylcupration-methylation methodology (Harris, et al., *Synthetic Approaches to Rapamycin. 3. Synthesis of a C1-C21 Fragment*, *Synlett*, pp. 903-905 (1996)) can be used in order to introduce the trisubstituted olefin. Thus the O-TBS ether 16a (Scheme 7) of propargylic alcohol 16 on treatment with the stannylcuprate reagent 20 followed by methylation with iodomethane provides the corresponding stannane which is then coupled under Stille conditions with the bromothiazole 17 to yield the olefin 19.

30

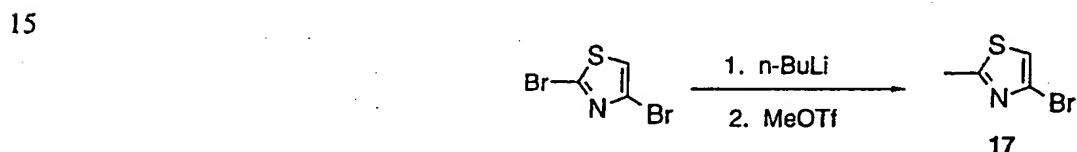
-16-

Scheme 7



10 The synthesis of 2-methyl-4-bromothiazole 17 from the known 2,4-dibromothiazole (Reynaud, et al., *Sur une Nouvelle Synthese du Cycle Thiazolique*, Bull. Soc. Chim. Fr. , 295:1735-1738(1962)) is outlined in Scheme 8

Scheme 8



20 The zirconium-catalyzed methylalumination strategy constitutes a novel route to construct the C16-C17 double bond and to introduce the thiazole ring. The novelty lies in the use of a chiral propargylic alcohol like 16 in the carbometalation reaction followed by the direct introduction of the thiazole unit.

This methodology also allows for the introduction of various substituents and chain elongations on the C12-C20 segment A. Thus starting with analogs of the ketone 15 in Scheme 6, a variety of chain-elongated derivatives of segment A can be produced. Also carrying out an ethylalumination (Et_3Al) in place of methylalumination (AlMe_3) (Scheme 6) allows the introduction of an ethyl group (Et) at C16. In the same context, other groups can also be introduced using the alternate stannylcupration-alkylation method by replacing iodomethane with other electrophiles in this reaction shown in Scheme 7. In addition, the thiazole ring can be replaced by other cyclic, aromatic and heteroaromatic rings by using other vinyl or aromatic/heteroaromatic halides in place of 2-methyl-4-bromothiazole 17 in the coupling reaction following either the carboalumination or stannylcupration strategy exemplified in Schemes 6 and 7 respectively.

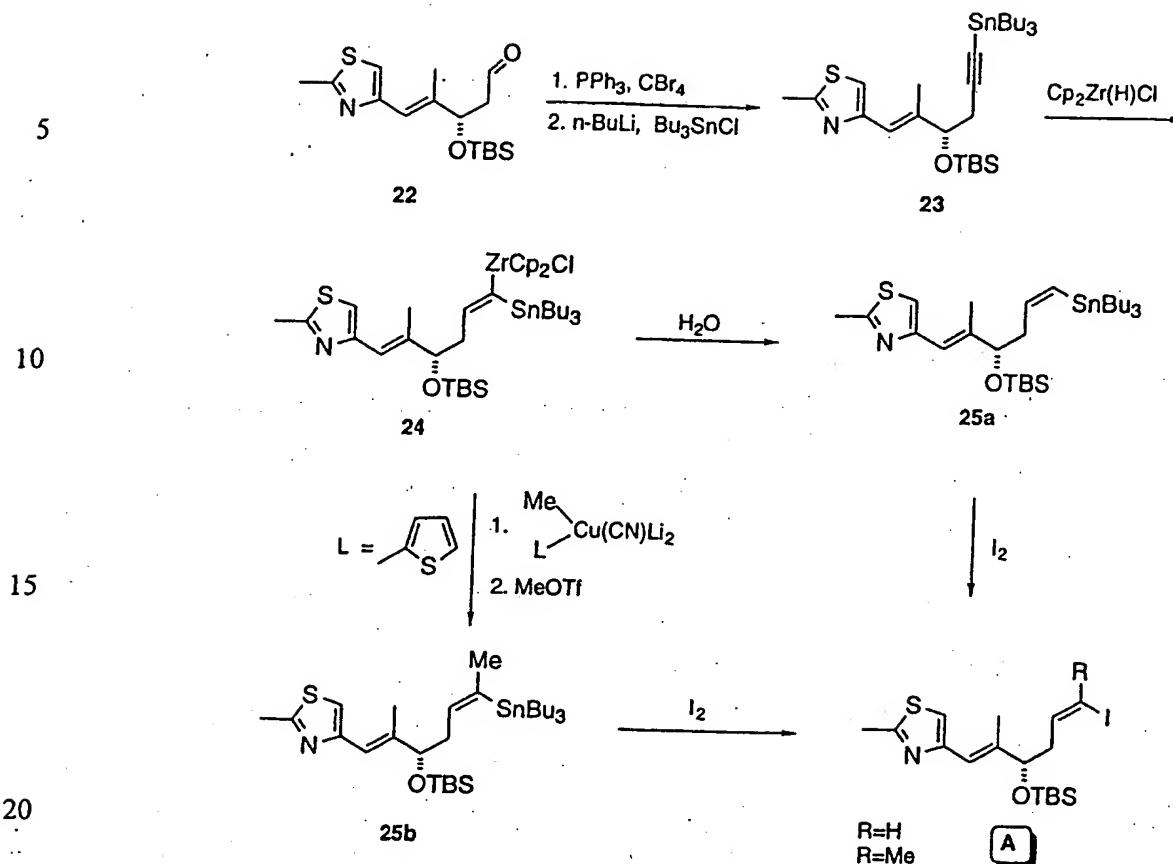
Stereoselective Construction of the C12-C13 cis-Olefinic Bond of Formula A

The goals in the construction of the C12-C13 Z-olefinic, were to design a method providing maximum control over the olefin geometry and to furnish common intermediates in the synthesis of both epothilones A and B. The introduction of affinity labels at C-12 was also a consideration.

The C12-C13 olefin can be constructed in the form of Z-vinyl iodides I that can be obtained from vinylstannanes with defined configurations. The vinyl stannanes will be accessed by using known chemistry reported by Lipshutz et al., *Preparation of Z-Vinylstannanes via Hydrozirconation of Stannylacetylenes*, Tetrahedron Lett., 33:5861-5864 (1992); Lipshutz, et al., *Hydrozirconation/Transmetalation of Acetylenic Stannanes. New 1,1-Dimetalloc Reagents*, Inorganica Chimica Acta, 220:41-44 (1994), which utilizes a 1,1-dimetalloc species as a stereodefined 1,1-vinyl dianion synthon. An exemplary synthesis is given in Scheme 9, for the precursor to a naturally occurring epothilone, and starts with a Corey-Fuchs reaction (PPh_3 , CBr_4) of the known aldehyde 22, followed by base-induced elimination and quenching of the lithium acetylide with tributyltin chloride (Bu_3SnCl) to yield alkynylstannane 23. The 1,1-dimetalloc species 24 is generated by hydrozirconation of the alkynyl stannane 23 using chlorohydrido zirconocene (Schwartz reagent). An aqueous quench would provide Z-vinylstannane 25a or alternatively, selective transmetalation with a higher order cuprate, followed by addition of an electrophile (MeOTf in case of epothilone B) to the resultant species provides the α -substituted vinylstannane 25b with high stereoselectivity. The Z-vinylstannanes 25a and 25b can then be transformed to the corresponding vinyl iodides I utilizing iodine with retention of configuration.

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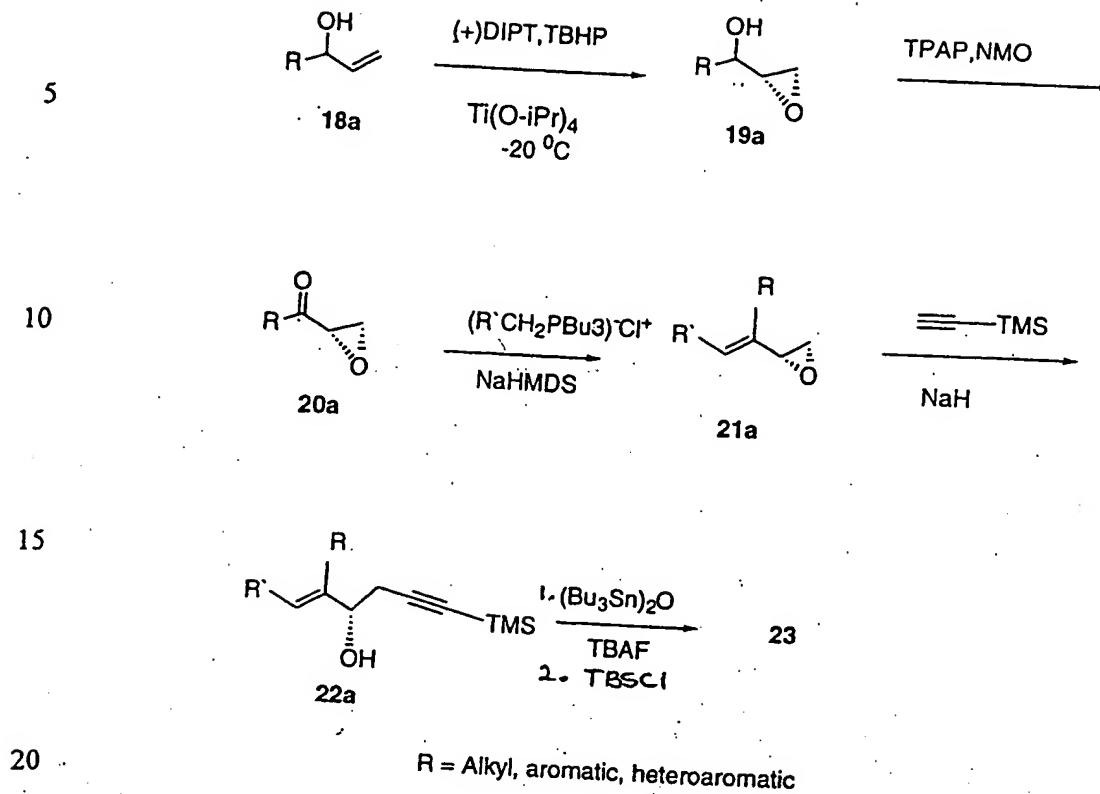
Scheme 9



25 An alternative route to the synthesis of alkynylstannane 23 (Scheme 9a) which would allow for incorporation of different substituents at the C16 carbon involves the asymmetric epoxidation of secondary alcohol 18a under the Sharpless conditions using (-)-diisopropyl tartrate, *tert*-butyl hydroperoxide and titanium isopropoxide to give epoxide 19a. The alcohol function on the epoxide can be oxidized with TPAP, NMO to give ketone 20a which can be reacted with Wittig reagents containing thiazole or other aromatic/heteroaromatic rings to give the corresponding *trans*-olefins. The terminal epoxide in this olefin can then be opened with trimethylsilyl acetylide to give secondary alcohol 22a. The trimethylsilyl group can then be substituted for a trialkyl stannyll group on treatment of 22a with TBAF and bis-tributyltin oxide and the obtained product treated with TBSCl to give compound 23.

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Scheme 9a

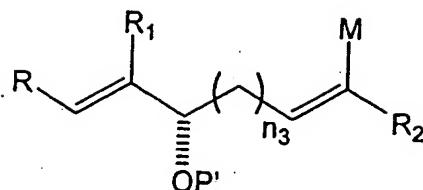


The foregoing chemistries can be used for the synthesis of analog precursors as well. Such analogs are best illustrated by Formula E, wherein n_3 , R, R₁ and R₂ are as defined previously. Again all of these modified segments can then be utilized in the total synthesis of various analogs of epothilones.

In summary, although the vinyl iodides E are previously reported (20,21) compounds, the method to synthesize it from the known aldehyde 22 is different from conditions reported in other total syntheses of epothilones. In addition, the above mentioned hydrozirconation reactions provide precise control over the geometry of the C12-C13 olefin bond. Also the use of other electrophiles in the transmetalation reaction with the intermediate species 24 allows for the synthesis of various analogs.

-20-

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FORMULA E

Two other epothilone derivatives of special interest may be synthesized in accordance with the invention. In one such derivative the lactone functional group is replaced with an ether functionality and in the other a lactam functionality is used in lieu of the lactone functional group. Thus in the first derivative, and referring to Formula A, X is O, Y is H₂, n₁, n₂, and n₃ are 1, R is 2-methyl-thiazol-4-yl, R₁ is methyl, R₂ is H or methyl, and R₃, R₄, R₅ and R₆ are methyl. In the second derivative, the only change is that X is NH and Y is O. These could be synthesized by the reaction sequences shown in Schemes 10 and 11. Thus selective deprotection at C1 by camphorsulfonic acid (CSA) (Scheme 10), formation of the mesylate derivative of the corresponding primary alcohol, selective deprotection of the C15 TBS ether and base-induced cyclic ether formation should provide compounds 26'. Again, the final stages in the synthesis would involve the deprotection of both the TBS groups from the macrolides (TFA, CH₂Cl₂) and the diastereoselective epoxidation of the C12-C13 double bond with epoxidizing agents such as dimethyldioxirane to give the ether derivatives 29 and 30.

20

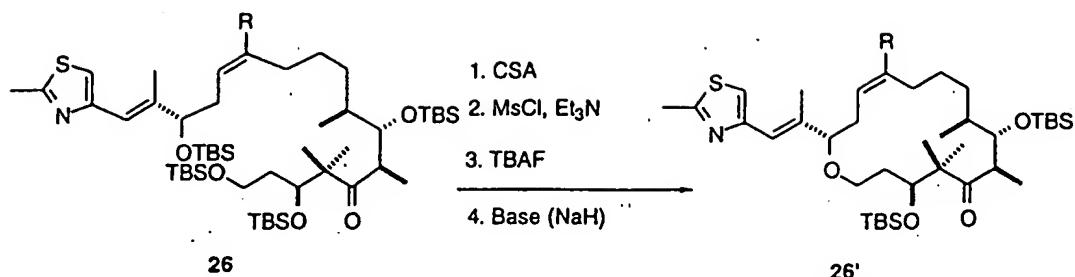
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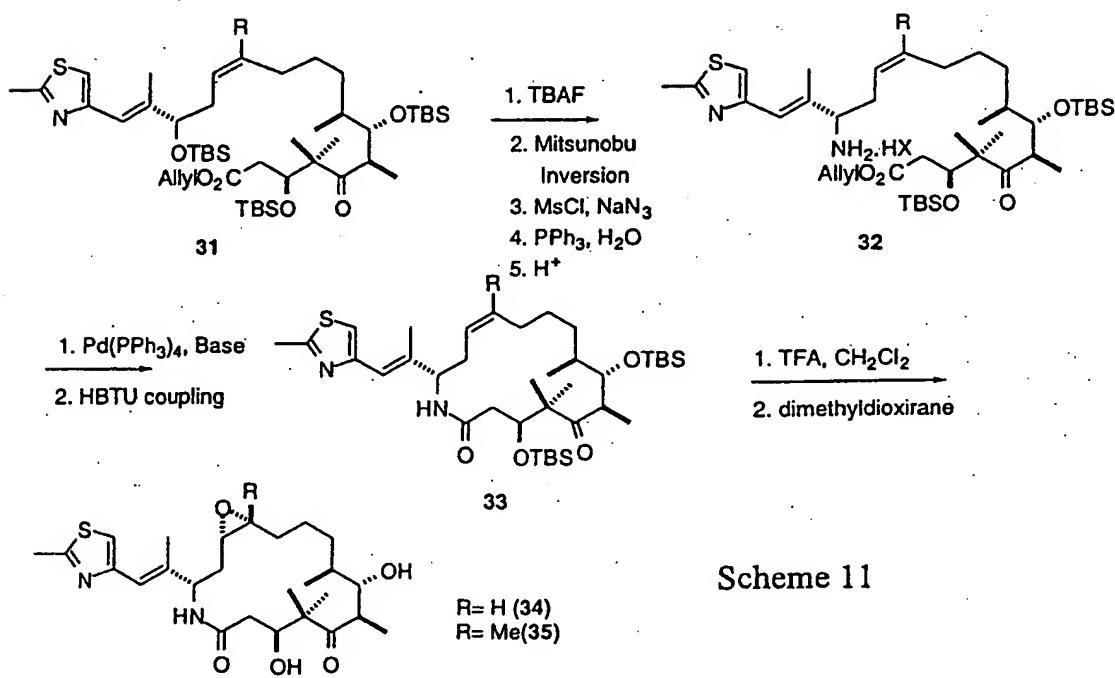
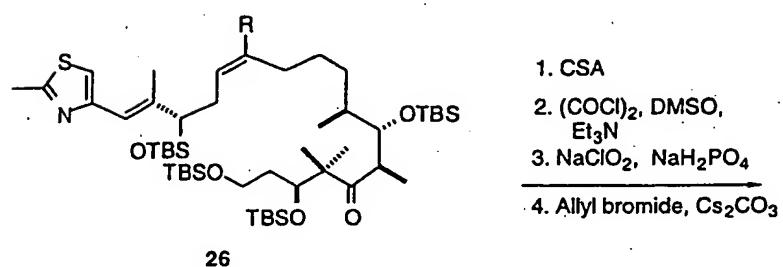
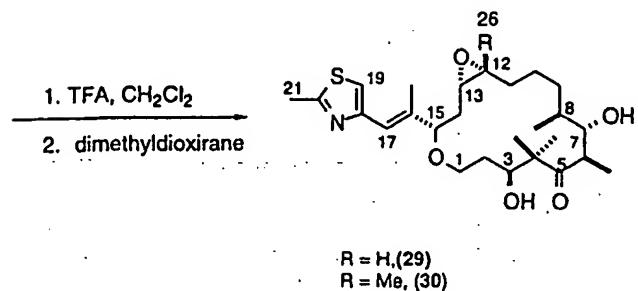
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For the lactam formation (Scheme 11) again compound 26 could be selectively deprotected at C-1 followed by sequential oxidation of the primary alcohol first under Swern conditions followed by NaClO₂-NaH₂PO₄ would furnish the known acids. These known acids can be converted to their allyl esters and then the TBS ether at C15 can be deprotected selectively. Mitsunobu inversion of these alcohols and azide formation via the corresponding mesylates will provide the azides with the correct stereochemistry at C15. Reduction of the azides (PPh₃, H₂O) followed by salt formation of the amine will provide 32. Deprotection of the allyl esters (Pd(PPh₃)₄, base) followed by macrolactamization (HBTU) will provide the lactams 33. Again, deprotection of both the TBS groups from the macrolides (TFA, CH₂Cl₂) and the diastereoselective epoxidation of the C12-C13 double bond with epoxidizing agents such as dimethyldioxirane would give the lactam derivatives 34 and 35.

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Scheme 10



Scheme 11

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Representative C4-C8 cycloalkyl, substituted and unsubstituted aromatic and heteroaromatic groups, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, C1-C10 alkoxy groups, and heterocyclic groups useful in the formation of epothilone analogs are set forth below.

5

C4-C8 cycloalkyl groups: cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl.

10

Substituted and unsubstituted aromatic groups: phenyl, phenyl groups substituted at any position with C1-C4 straight or branched chain alkyls, C1-C4 alkoxy groups, halogens, amines, amides, azides, sulfides, carboxylic acids and their derivatives, and hydroxides.

15

Substituted and unsubstituted heteroaromatic groups: thiazoles, pyrroles, furans, thiophenes, oxazoles and pyridines, and imidazoles.

C1-C10 straight and branched chain alkyl groups: methyl, ethyl, propyl, butyl, isopropyl, isobutyl, isopentyl, octyl, nonyl, and t-butyl.

20

Substituted and unsubstituted benzyl groups: benzyl, benzyl groups substituted at any position with C1-C4 straight or branched chain alkyls, C1-C4 alkoxy groups, halogens, amines, amides, azides, sulfides, carboxylic acids and their derivatives, and hydroxides.

25

C1-C10 alkoxy groups: methoxy, ethoxy, propoxy, butoxy, isopropoxy, t-butoxy, and nonoxy.

Heterocyclic groups: piperidines, furans, pyrroles, oxazolines, and thiophenes.

25

The following examples set forth various syntheses of the type described previously. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

EXAMPLE 1

30

The synthesis of segment C was accomplished via two unique and complementary routes which are detailed in Schemes 2 and 3. One novel step in the synthesis of the C1-C6 segment utilizes Noyori hydrogenation as detailed in Noyori, R. et al., *Asymmetric Hydrogenation of β -Keto Carboxylic Esters. A Practical, Purely Chemical Access to β -Hydroxy Esters in High Enantiomeric Excess*, 109 J. Am. Chem. Soc., pp. 5856-5858 (1987). The Noyori hydrogenation of β -keto ester 4 in Scheme 2 generates the requisite stereochemistry at C3. This Noyori hydrogenation provides

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the required enantiomer with high selectivities (92% enantiomeric excess). It is a versatile reaction and has found numerous applications in the synthesis of biologically active natural products and is also amenable to large scale synthesis.

The following is a detail of the procedures that are outlined in Scheme 2. The required β -keto ester 4 is obtained in two steps from the readily available starting material 3-benzyloxypropionic acid (2) as described by Davis et al., *Nonracemic α -fluoro aldehydes: Asymmetric synthesis of 4-deoxy-4-fluoro-d-arabinopyranose*, J. Org. Chem., 62:7546-7547 (1997), the teachings of which are hereby incorporated by reference. Isopropylcyclohexylamine (7.3 mL, 44.6 mmol, 1.5 eq.) was dissolved in 40 mL THF. The temperature was then lowered to -30°C and n-butyllithium (16.1 mL, 38.6 mmol, 1.3 eq.) was added dropwise and stirred for 30 minutes. Next, the temperature was raised to 0°C for 15 minutes and then cooled to -78°C for 15 minutes. Methyl isobutyrate (3.75 mL 32.7 mmol, 1.1 eq.) was dissolved in 5 mL THF and added dropwise. The resulting mixture was stirred 30 minutes. 3-Benzyloxypropionyl chloride (5.0 g, 29.7 mmol, 1 eq) in 5 mL THF was then added dropwise. This reaction mixture was stirred for one hour until the starting material completely disappears by thin layer chromatography (TLC) (80:20 hexanes/EtOAc). The reaction mixture was then quenched with 20 mL 20% HCl and raised to room temperature. Next, the reaction mixture was extracted 3 times with ether and the combined organic phases were washed twice with sodium bicarbonate and once with brine. The combined aqueous layers were cross-extracted twice with ether. The organics were combined, dried with Na_2SO_4 , and concentrated under reduced pressure. Purification was achieved via column chromatography utilizing a silica gel, hexane/EtOAc gradient which resulted in 5.1g (65% yield) of β -keto ester 4.

Asymmetric hydrogenation of β -keto ester 4 in methanol using $\text{RuBr}_2(S)\text{-binap}$ as catalyst at 65 psi gave the β -hydroxyester 5 in 85% yield. This was done by the following process. Acetone and MeOH were distilled and stored over molecular sieves. Each was degassed five times using the freeze-thaw method and placed under argon. Noyori's Ruthenium catalyst (91 mg, 0.284 mmol, 1 eq.), preferably bis-(2-methylallyl)cycloocta-1,5-diene ruthenium (II) and (S)-BINAP ((s)-(-)-1,1'-bi-2naphthol) (177 mg, 0.284 mmol, 1 eq.) were combined in a Schlenk flask with 24 mL acetone and 2.0 mL HBr solution (0.25 mL 48% HBr, 5.1 mL acetone). The resulting mixture was stirred for 4 hours to allow the catalyst to form. The acetone was then removed under reduced pressure. Next, beta ketoester 4 (5.36 g, 20.3 mmol, 71.5 eq.) in 23 mL MeOH was degassed four times and then transferred to a Parr hydrogenation

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flask using MeOH. The catalyst was then rinsed into the Parr flask using MeOH. The hydrogenation reaction was conducted over 110 hours at 65 psi and 60°C. The contents were concentrated under reduced pressure and then taken up in ether. The reaction mixture was filtered twice to remove the catalyst and then concentrated. Final purification was obtained through column chromatography wherein the column contained silica gel and utilized a hexane/EtOAc gradient and yielded 4.57 g (85% yield).

Deprotection of the benzyl ether and bis-silylation of diol 6 provided ester 7. To produce diol 6, β -hydroxyester 5 (1.824 g, 6.86 mmol, 1 eq.) was dissolved in 20 mL THF and transferred to a Parr hydrogenation vessel under argon. Pd(OH)₂ (450 mg, 0.25 eq.) was added and the flask purged for an additional 10 minutes with argon. The hydrogenation reaction was conducted at 50 psi for 24 hours. Finally, the reaction mixture was washed through a frit, an ultrafine strainer in a filtration technique preferably frit with 300 mL EtOAc and concentrated under reduced pressure to give 1.18g (90% yield) of diol 6.

In detail, ester 7 was made by taking diol 6 (450 mg, 2.84 mmol, 1 eq) dissolved in 3.15 mL DMF and adding imidazole (1.16 g, 17.04 mmol, 6 eq) which was stirred until dissolved. TBSCl (tert-Butyldimethylsilyl chloride) (1.28 g, 8.52 mmol, 3 eq) was added and the temperature was raised to 60°C. This reaction mixture was then stirred for 44 hours. Disappearance of the starting material was monitored by thin layer chromatography. The reaction was then quenched with H₂O and NH₄Cl. After quenching, the reaction mixture was extracted twice with ether. The ether layer was washed with NaHCO₃ and brine, dried with Na₂SO₄, and concentrated. Purification via column chromatography (SiO₂, 95:5 hexane/EtOAC) gave 0.89g or a 78% yield.

Primary alcohol 8 is the result of reducing ester 7 (1.89g, 4.68 mmol, 1 eq) by dissolving it in 26 mL of CH₂Cl₂, cooling it to -78°C and adding DIBAL-H (9.4 mL, 14 mmol, 1.5M in hexanes) dropwise. After stirring at this temperature (-78°C) for one hour, the reaction was quenched with 10 mL of MeOH. Next, the reaction mixture was warmed to 0°C and 10 mL of a saturated aqueous solution of potassium sodium tartarate was added. After stirring this mixture for 16 hours, the aqueous layer was extracted four times with CH₂Cl₂. The combined organics were dried over anhydrous sodium sulfate and concentrated to yield 1.59g (92% yield) of the primary alcohol. Although this is a known alcohol, the synthetic route from this ester is novel.

Primary alcohol 8 was oxidized to aldehyde 9 by taking primary alcohol 8 (1.45g, 3.86 mmol, 1 eq) and dissolving it in 25 mL CH₂Cl₂. Molecular sieves (4 Å,

-25-

powdered) were added to aid in the removal of water and this mixture was stirred for 15 minutes. 4-methylmorpholine N-oxide (NMO) (0.77g, 6.56 mmol, 1.7 eq) was then added and after stirring for 30 minutes, tetrapropylammonium perruthenate (TPAP) (0.081g, 0.23 mmol, 0.06 eq) was added. The reaction mixture was stirred for 16 hours at room temperature and then concentrated. It was then passed through a pad of 4:1 silica gel:Celite mixture (35g) to yield 1.2g (83% yield) of the aldehyde.

The aldehyde 9 is then reacted with EtMgBr using the procedure of Claus, E. et al., *Synthesis of the C1-C9 Segment of Epothilones*, 38 Tetrahedron Lett., 1359-1362 (1997), the procedure of which is hereby incorporated by reference, to give the known secondary alcohol 10 in 65% yield.

This secondary alcohol 10 is then oxidized to the C1-C6 segment C using the same procedure that was used to oxidize primary alcohol 8 to aldehyde 9. The alcohol 10 (50 mg, 0.124 mmol, 1 eq) was dissolved in 1 mL CH₂Cl₂. Molecular sieves (4 Å, powdered) were added and this mixture was stirred for 15 minutes. N-oxide NMO (25 g, 0.211 mmol, 1.7 eq) was then added and after stirring for 30 minutes, TPAP (3 mg, 0.0074 mmol, 0.06 eq) was added. The reaction mixture was stirred for 15 hours at room temperature and then concentrated. It was then purified by passing it through a column (20 g) of 5:1 silica gel:Celite mixture (5% EtOAC in hexane) to yield 46 mg (92%) of the ketone (segment C). Again although segment C is known the oxidation process used is different from conditions reported.

In summary, although segment C is a key synthon in previously reported total syntheses of the epothilones, the synthetic route utilizing the asymmetric Noyori hydrogenation is unique.

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EXAMPLE 2

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Scheme 3 outlines an alternate synthesis of β-hydroxyester 5 using known compound 13. This alternate route toward segment C allows for the introduction of affinity labels and modifications at the C4 position as shown in Scheme 3. Applying the Noyori reduction to the known unsubstituted β-keto ester 11 provides a building block that can be used for the modifications at C4 of the epothilones. There has only been one report so far of C4 modification on the epothilones and this method provides a more general route of introducing a variety of substituents at this position. This will also enable a more thorough study of the structure activity relationships of numerous C4 substituted analogues.

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Thus, the Noyori hydrogenation of β -keto ester 11 yields the known β -hydroxy-ester 12, which was reported by Ali, et. al, *Formal Syntheses of Cryptophycin 1 and Arenastatin A.*, 38 Tetrahedron Lett, 1703-1706 (1997), hereby incorporated by reference, in 97% yield (in 97% enantiomeric excess). The Frater alkylation of β -hydroxy ester 12 yields the previously reported α -methyl analogue 13 also previously reported by Ali et al. *Formal Syntheses of Cryptophycin 1 and Arenastatin A.*, 38 Tetrahedron Lett, 1703-1706 (1997), hereby incorporated by reference, in 71% yield (98% diastereomeric excess). A second Frater alkylation of hydroxy ester 13 gave bis-dimethyl derivative 5 in 59% yield which was then converted to epothilone segment C by the chemistry shown in Scheme 2.

In detail, isopropylcyclohexylamine (0.71 mL, 4.32 mmol, 2.16 eq.) and 3.75 mL THF were stirred together at -25°C. *n*-BuLi (1.64 mL, 3.6 mmol, 1.8 eq.) was added dropwise over 15 minutes. The reaction mixture was stirred at room temperature for 15 minutes and then lowered to -78°C. Compound 13 (504 mg, 2 mmol, 1 eq.) in 2.5 mL THF was added to the reaction mixture. The temperature was gradually raised to -10°C over 4 hours and then returned to -78°C. MeI (0.17 mL, 2.66 mmol, 1.33 eq.) in HMPA (0.26 mL) was added dropwise. The reaction was stirred at -78 °C for one hour and then stirred at room temperature for 16 hours. The reaction was quenched with 4 mL 10% HCl. The mixture was then extracted four times with CH₂Cl₂, dried over Na₂SO₄, and concentrated. Purification by column chromatography 160 g SiO₂ (hexane/EtOAc gradient) gave 312 mg (a 59% yield) of β -hydroxyester 5.

At this stage, other substituents such as benzyl, allyl and other alkyl groups can be introduced by using other electrophiles in the second Frater alkylation in place of iodomethane. The novel aspect about this alternate route to segment C is the ability to alter the substituents at the C4 position of the epothilones using the aforementioned Frater alkylation strategy. After synthesis of β -hydroxyester 5, segment C can be produced following the procedure outlined above in Scheme 2.

It should be noted that this invention makes it possible to synthesize several analogs of this C1-C6 segment with various chain elongations at C2 and substitution at C6 positions on the epothilones. It also allows for, as mentioned before, modifications at the C4 position with other groups such as aryl, heterocyclic, alkyl and branched alkyl. These chain extensions and substitutions are illustrated by Formula C. The synthesis of these modified derivatives can be achieved utilizing chemistry exemplified in the synthesis of segment C in Schemes 2 and 3 respectively. These

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modified segments can then be utilized in the total synthesis of various analogs of epothilones.

EXAMPLE 3

This example illustrates the synthesis of segment B. The synthesis of the C7-C11 segment B has been achieved using previously reported chemistry of Lin, et al., *Efficient Total Syntheses of Pumiliotoxins A and B. Applications of Iodide-Promoted Iminium Ion-Alkyne Cyclization in Alkaloid Construction* 118 J. Am. Chem. Soc., 9062-9072 (1996), the teachings of which are hereby incorporated by reference, and is outlined in Scheme 4. This synthesis can also be used to introduce various chain-elongations on this segment and to introduce various other substituents at C-8. These modifications can be illustrated by Formula D and their synthesis can be achieved using chemistry exemplified in the synthesis of segment B in Scheme 4. Again, these modified segments can then be utilized in the total synthesis of various analogs of epothilones.

EXAMPLE 4

This example, illustrated in Scheme 5, describes the synthesis of aldol adduct 14 followed by the aldol reaction of segment C, from examples 1 or 2, with segment B, from example 3. Aldol adduct 14 was then used to synthesize segment D.

The connection of the two segments C and B utilizes a highly diastereoselective aldol reaction (Scheme 5). When the C1-C6 ketone segment C was treated with a base, namely lithium diisopropylamide and the resultant enolate reacted with C7-C11 aldehyde segment B a single diastereomer 14 was observed in 21% yield (unoptimized). The remarkable diastereoselectivity is speculated to arise from a favorable nonbonding interaction between the C10-C11 double bond and the carbonyl group of the aldehyde that gives rise to the desired diastereomer. This connection between these two particular segments using an aldol reaction is unprecedented. After the connection has been made, the resultant secondary alcohol will be protected as the corresponding *tert*-butyldimethylsilyl ether D.

In detail, diisopropylamine (60 μ L, 0.45 mmol, 1 eq) dissolved in 1 mL THF was cooled to -78 °C and n-BuLi (0.33 mL, 0.43 mmol, 0.95 eq) was added dropwise. After stirring at -78 °C for 15 minutes and at 0 °C for 30 minutes the reaction mixture was recooled to -78 °C. The ketone (segment C) (0.184g, 0.45 mmol, 1 eq) dissolved in 1 mL of THF was then added dropwise. This mixture was stirred at -78 °C for 15

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5 minutes and then warmed to -40°C over one hour. After recooling to -78°C, the aldehyde segment B (0.022g, 0.23 mmol, 0.5 eq) dissolved in 0.5 mL Et₂O was added dropwise over 15 minutes. After 35 minutes at -78°C, the reaction was quenched with 2 mL saturated aqueous ammonium chloride and warmed to room temperature. The aqueous layer was extracted five times with Et₂O and the combined organics were dried over anhydrous magnesium sulfate. After concentration, preparative thin layer chromatography of the residue yielded 0.047g (21% yield) of the desired diastereomer.

10 Forming segment D utilizes TBS protection of adduct 14. Aldol adduct 14 (30 mg, 0.06 mmol, 1 eq.) was diluted with 1.5 mL CH₂Cl₂ and the temperature was lowered to -78 °C. 2,6-lutidine (50 μL, 0.42 mmol, 7 eq.) was added dropwise followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (70 μL, 0.30 mmol, 5 eq.). The reaction was stirred for 15 minutes and then raised to 0 °C. The reaction was complete after 3 hours and was quenched with 5 mL NH₄Cl. The mixture was extracted three times with CH₂Cl₂ and the combined organic layers were washed with brine, dried over MgSO₄ and then concentrated. Column chromatography (5% EtOAc in hexane) gave 34 mg (96% yield) of compound D.

EXAMPLE 5

20 This example illustrates the synthesis of segment A (the C-12 - C-20 segment) and is outlined in Scheme 6. This involves new ways to set the C16-C17 trisubstituted double bond and the C12-C13 *cis*-double bond which serves as precursor to the *cis*-epoxide at C12-C13 in the epothilones. The methodology used to introduce the thiazole moiety draws upon zirconium-catalyzed carboalumination chemistry as described by Wipf, P. and Lim, S., *Rapid Carboalumination of Alkynes in the Presence of Water*, 32 Agnew. Chem., Int. Ed. Engl., 1068-1071 (1993), the teachings of which are hereby incorporated by reference. Using this chemistry, a C16-C17 alkyne bond in an appropriately functionalized C13-C17 propargylic alcohol 16 is subjected to methylalumination in the presence of zirconocene dichloride (Cp₂ZrCl₂). The chiral propargylic alcohol 16 is obtained via the asymmetric reduction of the readily available alkynyl ketone 15. A number of methods have been developed during the past years for the enantioselective reduction of α,β-alkynyl ketones. The resultant alkenylalane is coupled with 2-methyl-4-bromothiazole 17 in the presence of zinc chloride under Pd(0) catalysis as described by Negishi, E.-I., et al., in *Double Metal Catalysis in the Cross-Coupling Reaction and Its Application to Stereo- and Regioselective Synthesis of Trisubstituted Olefins*, 100 J. Am. Chem. Soc., 2254-2256 (1978) and Negishi, E.-I.,

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in *Palladium- or Nickel-Catalyzed Cross Coupling: A New Selective Method for Carbon-Carbon Bond Formation*, 15 Acc. Chem. Res., 340-348 (1982), the teachings of which are hereby incorporated by reference. Scheme 8 illustrates the synthesis of 2-methyl-4-bromothiazole 17 which was synthesized by adding 9.0 mL ether and *n*-BuLi (1.8 mL, 2.96 mmol, 1.2 eq) and stirring at -78°C for 30 minutes. The bromothiazole (0.60 g, 2.47 mmol, 1 eq.) in 3.5 mL of ether was added dropwise to the *n*-BuLi. After stirring for one hour, MeOTf (0.56 mL, 4.94 mmol, 2 eq.) was added dropwise and the reaction mixture was stirred for an additional 1.5 hours. The reaction was then quenched with NaHCO₃ and extracted 5 times with ether. The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated. 262 mg (60% yield) of the 2-methyl-4-bromothiazole 17 was obtained and carried on without further purification.

This coupling of the alkenylalane to 2-methyl-4-bromothiazole 17 permits access to the trisubstituted *E*-olefin 19 stereoselectively following the protection of the alcohol 18 as the OTBS-ether.

After the introduction of the thiazole moiety, the known primary alcohol 21 (Mulzer et al., *Easy Access to the Epothilone Family - Synthesis of Epothilone B*, Tetrahedron Lett., 39:8633-8636 (1998) is revealed by deprotection of the PMB ether 19 and then oxidized to C13-C20 aldehyde 22, also previously reported by Mulzer, et al., *Easy Access to the Epothilone Family - Synthesis of Epothilone B*, 39 Tetrahedron Lett., 8633-8636 (1998), the teachings of which are hereby incorporated by reference.

The zirconium-catalyzed methylalumination strategy constitutes a novel route of constructing the C16-C17 double bond and introduction of the thiazole ring. The novelty lies in the unprecedented use of a chiral propargylic alcohol like 16 in the carbometalation reaction followed by the direct introduction of the thiazole unit.

This methodology also allows for the introduction of various substituents and chain elongations on the C12-C20 segment A. Thus starting with analogs of the ketone 15 in Scheme 6, a variety of chain-elongated derivatives of segment A can be accessed. Also carrying out an ethylalumination (Et₂Al) in place of methylalumination (AlMe₃) will allow the introduction of an ethyl group (Et) at C16. In the same context, other groups such as alkyl and benzyl groups can also be introduced using the alternate stannylcupration-alkylation method by replacing iodomethane with other electrophiles in this reaction shown below in Example 6 and illustrated in Scheme 7. In addition the thiazole ring can be replaced by other cyclic, aromatic and heteroaromatic rings by using other vinyl or aromatic/heteroaromatic halides in place of 2-methyl-4-bromothia-

-30-

sole 17 in the coupling reaction following either the carboalumination or stannylcupration strategy exemplified in Schemes 6 and 7 respectively.

These aforementioned modifications are best illustrated by Formula E. Again all of these modified segments can then be utilized in the total synthesis of various 5 analogs of epothilones.

EXAMPLE 6

Scheme 7 illustrates an alternative way to introduce the trisubstituted olefin. This method utilizes the stannylcupration-methylation methodology described by 10 Harris, L., et al., in *Synthetic Approaches to Rapamycin 3. Synthesis of a C1-C21 Fragment*, Synlett, 903-905 (1996), the methodology of which is hereby incorporated by reference. Thus the O-TBS ether 16a from Scheme 7 of propargylic alcohol 16 on treatment with the stannylcuprate reagent 20 followed by methylation with iodomethane would provide the corresponding stannane which would then be coupled under Stille 15 conditions with the bromothiazole 17 to yield the olefin 19.

EXAMPLE 7

The synthesis of 2-methyl-4-bromothiazole 17 from the known 2,4-dibromothiazole is outlined in Scheme 8. This 2,4-dibromothiazole has been previously reported 20 by Reynaud, P., Robba, M. and Moreau, R. C. in *Sur une Nouvelle Synthese du Cycle Thiazolique*, 295 Bull. Soc. Chim. Fr., 1735-1738 (1962), the teachings of which are hereby incorporated by reference.

EXAMPLE 8

This example illustrates the stereoselective construction of the C12-C13 *cis*-olefinic bond, the process of which is outlined in Scheme 9. The method shown provides maximum control over the olefin geometry as well as furnishes common intermediates in the synthesis of both epothilones A and B. This method is also amenable to introduction of affinity labels at C-12.

The C12-C13 olefin is constructed in the form of Z-vinyl iodides A that can be obtained from vinylstannanes with defined configurations. The vinyl stannanes will be accessed by using the chemistry reported by Lipshutz et al., in *Preparation of Z-Vinylstannanes via Hydrozirconation of Stannylacetylenes*, 33 Tetrahedron Lett., 5861-30 5864 (1992) and Lipshutz, B. H. and Keil, R. in *Hydrozirconation/Transmetalation of Acetylenic Stannanes. New I,I-Dimetalloc Reagents*, 220 Inorganica Chimica Acta, 41-

-31-

44 (1994), the teachings of which are hereby incorporated by reference. The chemistry utilizes a 1,1-dimetallo species as a stereodefined 1,1-vinyl dianion synthon.

The synthesis starts with a Corey-Fuchs reaction (PPh_3 , CBr_4), as described by Corey, E. J. and Fuchs, P. L., *A Synthetic Method for Formyl to Ethynyl Conversion*, Tetrahedron Lett., 36:3769-3772 (1972) of the known aldehyde 22, followed by base-induced elimination and quenching of the lithium acetylide with tributyltin chloride (Bu_3SnCl) to yield alkynylstannane 23. The 1,1-dimetallo species 24 is generated by hydrozirconation of the alkynyl stannane 23 using chlorohydridozirconocene (Schwartz reagent). An aqueous quench provides Z-vinylstannane 25a or alternatively, selective transmetalation with a higher order cuprate, followed by addition of an electrophile (MeOTf in case of epothilone B) to the resultant species provides the α -substituted vinylstannane 25b with high stereoselectivity. The Z-vinylstannanes 25a and 25b can then be transformed to the corresponding vinyl iodides A utilizing iodine with retention of configuration.

In summary, although the vinyl iodides A are previously reported compounds as evidenced by the teachings of Schinzer, D. et al., in *Total Synthesis of (-)-Epothilone A*, 36 Angew. Chem., Int. Ed. Engl., 523-524 (1997) and Schinzer, D., Bauer, A., and Scheiber, J., *Synthesis of Epothilones: Stereoselective Routes to Epothilone B*, Synlett, 861-864 (1998), the teachings of both are hereby incorporated by reference, the method to synthesize it from the known aldehyde 22 is different from conditions reported in other total syntheses of epothilones. In addition, the above mentioned hydrozirconation reactions provides precise control over the geometry of the C12-C13 olefin bond and this methodology constitutes a unique way to construct this double bond. Also the use of other electrophiles in the transmetalation reaction with the intermediate species 24 allows for the synthesis of various analogs modified at C12 position of the epothilones as illustrated in Formula E.

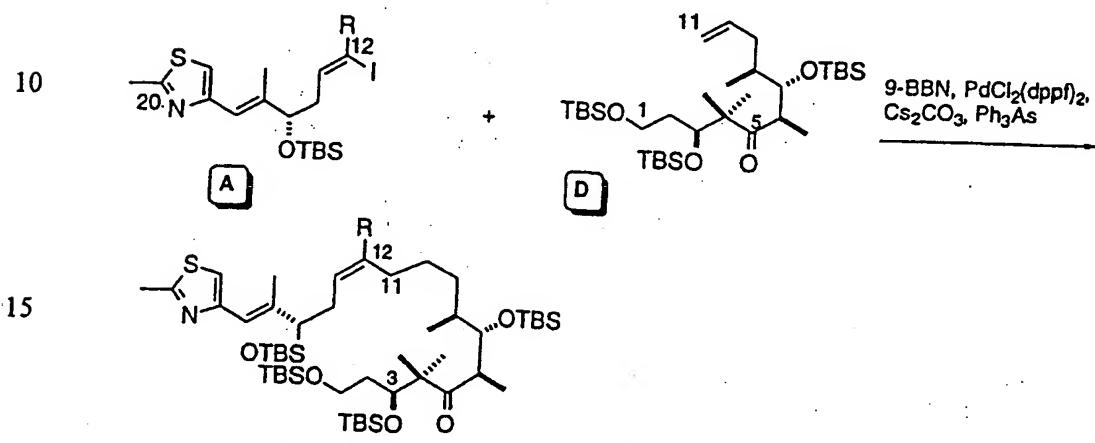
EXAMPLE 9

This example and the next (Example 10) illustrate C11-C12 bond construction including coupling of the C1-C11 (segment D) and C12-C20 (segment A) subunits and completion of the total synthesis.

Having defined all the requisite stereocenters and geometries, the stage is set for the union of the C1-C11 and the C12-C20 subunits. The C11-C12 bond connection is achieved by the B-alkyl Suzuki reaction of the C1-C11 olefin D with the vinyl iodides A shown in Scheme 10 to afford the precursors 26 for the synthesis of epothilone A and

-32-

B. This *B*-alkyl Suzuki reaction is described by Miyaura, N., and Suzuki, A. in *Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds*, 95 Chem. Rev. 2457-2483 (1995), the teachings of which are hereby incorporated by reference. Thus, hydroboration of the olefin D with 9-BBN followed by coupling of the corresponding organoborane with the vinyl iodides A in presence of $[\text{PdCl}_2(\text{dppf})_2]$ would yield the olefin 26.



Scheme 12

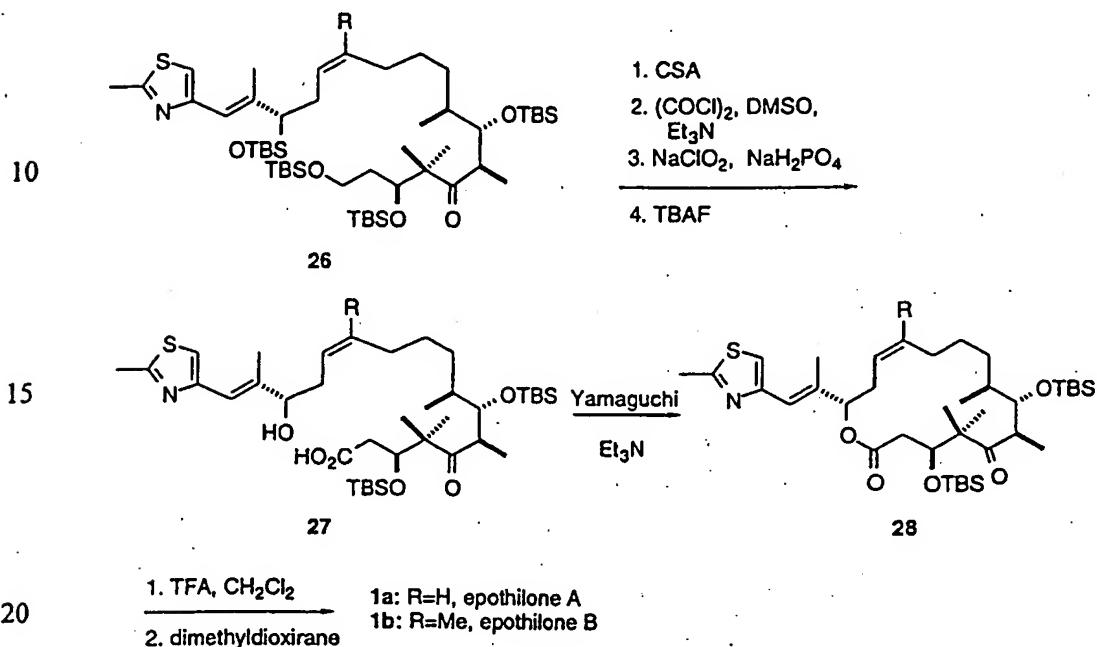
EXAMPLE 10

Finally the conversion of the coupled C1-C20 products 26 to epothilones A and B is accomplished using the previously reported procedures of Nicolaou, K. C. et al., in *Total Syntheses of Epothilones A and B via a Macrolactonization-Based Strategy* 119 J. Am. Chem. Soc., 7974-7991 (1997), the teachings of which are hereby incorporated by reference. Thus selective deprotection at C1 by camphorsulfonic acid (CSA), shown in Scheme 11, followed by sequential oxidation of the primary alcohol first under Swern conditions followed by NaClO₂-NaH₂PO₄ furnishes the known acids. Selective deprotection of the TBS ether at C15 using tetrabutylammonium fluoride yields the hydroxy acids 27. The key macrolactonization step is then carried out using the Yamaguchi method as described by Inanaga, J. et al., in *A Rapid Esterification by Means of Mixed Anhydride and its Application to Large-ring Macrolactonization*, 52 Bull. Chem. Soc. Jpn., 1989-1993 (1979), the teachings of which are hereby incorporated by reference, affording the known 16-membered macrolides 28. The final stages in the

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synthesis involve the deprotection of both the TBS groups from the macrolides 28 (TFA, CH₂Cl₂) and the diastereoselective epoxidation of the C12-C13 double bond with epoxidizing agents such as dimethyldioxirane or methyl(trifluoromethyl)dioxirane.

5



Scheme 13

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As shown in Formulae C, D and E, various modified segments can be employed during the syntheses of the individual segments (C, B and A). All of these modified segments can then be connected using the bond connections (Aldol reaction and the *B*-alkyl Suzuki reaction) highlighted in this total synthesis. This would provide numerous homologs, analogs and affinity labels of the epothilones. All references noted herein are expressly incorporated by reference..

30

Acronym and Symbol Definitions

In order to facilitate the preceding discussion various acronyms and symbols have been used. These have the following definitions.

5	Ac	acetyl
	Ar	aromatic
	9-BBN	9-borabicyclo[3.3.1]nonane
	(S)-BINAP	(S)-(-)-1,1'-bi-2-naphthal
	Bn	benzyl
10	BnO	benzyloxy
	Bu	butyl
	n-BuLi	n-butyl lithium
	Cp ₂ ZrCl ₂	zirconocene dichloride
	Cp ₂ Zr(H)Cl	chlorohydrido zirconocene
15	CSA	camphorsulfonic acid
	DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
	DIBAL-H	diisobutylaluminum hydride
	DIEA	diisopropylethylamine
	(+)-DIPT	diisopropyl tartrate
20	DMF	N,N-dimethylformamide
	DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)pyrimidinone
	DMSO	dimethylsulfoxide
	Et	ethyl
	HBTU	O-(benzotriazol-1-y-1)-N,N,N',N' tetramethyluronium hexafluoro phosphate
25	HF	hydrogen fluoride
	HMPA	hexamethylphosphoramide
	LDA	lithium diisopropylamide
	LHMDS	lithium hexamethyldisilazide
30	LICA	lithium isopropylcyclohexylamide
	m-CPBA	meta-chloroperoxybenzoic acid
	MDR	multi-drug resistant
	Me	methyl
	MeOTf	methyl triflate
35	MS	molecular sieves
	MsCl	mesyl chloride
	NaHMDS	sodium hexamethyldisilazide
	NMO	4-methylmorpholine N-oxide
	OTf	trifluoromethane sulfonate
40	PdCl ₂ (dpff) ₂	dichloro[1,1'-Bis(diphenylphosphino)ferrocene] palladium II
	Pd(PPh ₃) ₄	tetrakis (triphenylphosphine) palladium (0)
	Ph	phenyl
	PPh ₃	triphenylphosphine
	PMB	para-methoxybenzyl
45	Ru cat	bis-(2 methyl allyl) cycloocta-1,5-diene ruthenium (II)

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TBAF	tetrabutylammonium fluoride
TBHP	tertbutyl hydroperoxide
TBS	tertiary-butyldimethylsilyl
TBSCl	tertiary-butyldimethylsilylchloride
5 TBSOTf	tertiary-butyldimethylsilyl triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Ti(O-iPr) ₄	titanium isopropoxide
TPAP	tetrapropylammonium perruthenate

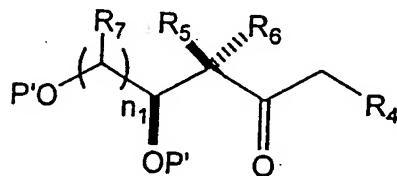
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We claim:

1. A method of synthesizing an epothilone precursor of the formula

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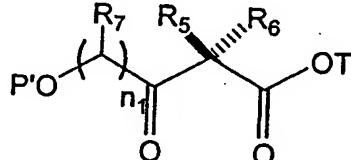


where n_1 is an integer from 0-4, R_4 is selected from the group consisting of H, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1-C10 alkoxy groups, R_5 and R_6 are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1-C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups, R_7 is H or straight or branched chain C1-C10 alkyl groups, and P' is a protective group, comprising the steps of:

15

providing a β -keto ester of the formula

20



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where n_1 , R_4 , R_5 , R_6 , R_7 , and P' are as defined above, and T is an alkyl group; preferentially hydrogenating the C3 keto group of said β -keto ester to form the corresponding hydroxyester by reacting the β -keto ester with a hydrogenating agent in the presence of an asymmetric organometallic molecular catalyst comprising a metal atom or ion having one or more chiral ligands coupled thereto; and converting the hydroxyester to said epothilone precursor.

30

2. The method of claim 1, said hydrogenating agent being hydrogen.

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3. The method of claim 1, said hydrogenating step being carried out at a pressure of from about 30-100 psi.

4. The method of claim 3, said pressure being from about 50-75 psi.

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5. The method of claim 1, said hydrogenating step being carried out at a temperature of from about 40-100°C.

6. The method of claim 5, said temperature being from about 50-
5 75°C.

7. The method of claim 1, said hydrogenating step being carried out for a period of from about 12 hours to 5 days.

10 8. The method of claim 7, said period being from about 2-5 days.

9. The method of claim 1, including the step of agitating the reaction mixture during said hydrogenation step.

15 10. The method of claim 1, said catalyst being present during said hydrogenation step at a level of from about 5 mol % to about 25 mol%.

11. The method of claim 1, said converting step comprising the steps
of:

20 removing the P' protecting group from said hydroxy ester to form a diol;
protecting the oxygen atoms of said diol to form a protected diol;
reducing the ester function of said protected diol to a primary alcohol;
oxidizing the primary alcohol to an aldehyde;
reacting the aldehyde with a Grignard reagent having said R₄ group coupled
25 thereto to form a secondary alcohol; and
oxidizing the secondary alcohol to form said epothilone precursor.

30 12. The method of claim 11, said P' removal step comprising the steps of reacting said hydroxyester with hydrogen in the presence of a catalyst at a pressure of from about 40-100 psi.

13. The method of claim 12, said catalyst selected from the group consisting of Pd(OH)₂ and Pd/C.

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14. The method of claim 11, said oxygen atom protecting step comprising the steps of reacting said diol with TBS chloride in a compatible solvent at a temperature of from about 40-100°C for a period of from about 30-60 hours.

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15. The method of claim 11, said ester function reducing step comprising the steps of reacting said protected diol with a reducing agent DIBAL-H at a temperature of from about -20 to -85°C.

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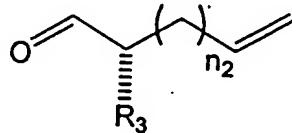
16. The method of claim 11, said primary alcohol oxidizing step comprising the steps of reacting the primary alcohol with 4-methylmorpholine N-oxide and a catalytic amount of tetrapropylammonium perruthenate.

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17. A method of connecting epothilone precursors comprising the steps of:

providing a first epothilone precursor of the formula

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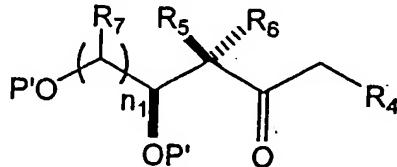


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where n_2 is an integer from 1-4, and R_3 is selected from the group consisting of H, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1-C10 alkoxy groups;

providing a second epothilone moiety of the formula

15



20

where n_1 is an integer from 0-4, R_4 is selected from the group consisting of H, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1-C10 alkoxy groups, R_5 and R_6 are each individually and respectively selected from the group consisting of H, substituted and unsubstituted aryl and heterocyclic groups, C1-C10 straight and branched chain alkyl groups, and substituted and unsubstituted benzyl groups; R_7 is H, or straight or branched chain C1-C10 alkyl groups, and P' is a protecting group, comprising the steps of: reacting said second epothilone precursor with a base to form an enolate; and reacting said enolate with said first epothilone precursor.

25

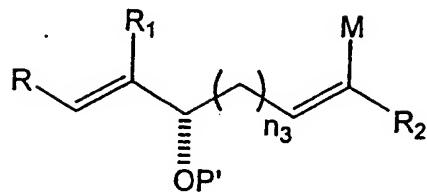
30 18. The method of claim 17, said enolate-forming and enolate-first epothilone reactions comprising the steps of initially cooling said base to a temperature of at least about -75°C, and adding said second precursor to the cooled base, slowly elevating the temperature of the base-second precursor mixture to about -40°C, and thereafter recooling the mixture to at least about -75°C and adding said first precursor thereto.

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19. A method of synthesizing a vinyl halide epothilone precursor having the formula

5

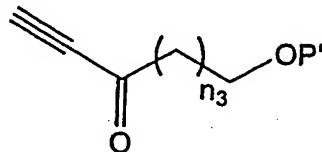


10 where n_3 is an integer from 1-4, R is selected from the group consisting of C4-C8 cycloalkyl, and substituted and unsubstituted aromatic and heteroaromatic groups, R₁ and R₂, are each individually and respectively selected from the group consisting of H, C1-C10 straight and branched chain alkyl groups, substituted and unsubstituted benzyl groups, and C1-C10 alkoxy groups, P' is a protecting group, and M is either bromine or iodine, comprising the steps of:

15

providing an alkynyl ketone of the formula

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wherein n_3 , M and R₂, are as previously defined; asymmetrically reducing said alkynyl ketone to create the alcohol form of the alkynyl ketone;

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reacting said alcohol form with a reagent system selected from the group consisting of (R₁)₃Al and zirconocene dichloride or stannyl cupration reagent and R₁-halide to form a vinyl metal species;

30

reacting said vinyl metal species with an aryl or vinyl halide to form an allyl alcohol; and

converting said allyl alcohol to said halide epothilone precursor.

20. The method of claim 19, said asymmetric reduction step comprising the step of creating the protected alcohol form of the alkynyl ketone.

35

21. The method of claim 19, said R₁-halide being selected from the group consisting of R₁Br and R₁I.

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22. The method of claim 19, said conversion step including the step of initially converting the allyl alcohol to an alkynyl stannane, reducing said alkynyl stannane with chlorohydridozirconocene to form a 1,1-dimetalloc Zr-Sn species.

5 23. The method of claim 22, including the steps of hydrating said dimetalloc species to form a vinyl stannane, and then quenching the vinyl stannane with either iodine or bromine.

10 24. The method of claim 22, including the steps of transmetallating said dimetalloc species with an organocuprate and then quenching with an alkyl-R₂-OTf, and thereafter quenching with either iodine or bromine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07765

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07C 45/00

US CL :568/309, 338, 383

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 568/309, 338, 383

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHAUHAN.K. Total Synthesis of the Ethanol Inducible, Proinflammatory Autoacoid 3(S)-Hydroxy-Leukotriene B4 (3-OH-LTB4) and Analogs; Tetrahedron Lett. 1994. Vol 35. No. 12. pages 1825-1828.	19-24
A	Kerdesky. F.A. Total Synthesis of Leukotriene B4; J.Organic Chemistry (1993). Vol 58. No. 13. pages 3516-3520	19-24
A	SHAO. L. Asymmetric Hydrogenation of 3,5-dioxo esters by Rabinap Complex: A Short Step Asymmetric of 6-Substituted 5,6-Dihydro-2-Pyrone; Tetrahedron (1993). Vol. 49. No. 10. pages 1997-2010.	1-24

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